POTASSIUM OXALATE AS A NITRIFICATION INHIBITOR AND ITS EFFECT ON MICROBIAL POPULATIONS AND ACTIVITIES IN A CALCAREOUS UZBEKISTANIAN SOIL UNDER COTTON CULTIVATION

M. Mamiev¹, D. Egamberdiyeva¹, D. Berdiev¹, and S. Poberejskaya²

¹Institute of Microbiology, Abdulla Qadiriy str. 7 B, 700128 Tashkent, Uzbekistan
²Institute of General and Inorganic Chemistry, 700170 Tashkent, Uzbekistan

Corresponding author’s e-mail: dilfuza_egamberdiyeva@yahoo.com

ABSTRACT
Application of fertilizers combined with nitrification inhibitors affects soil microbial biomass and activity. The objective of this research was to determine the effects of fertilizer application combined with the nitrification inhibitor potassium oxalate (PO) on soil microbial population and activities in a nitrogen poor soil in Uzbekistan under cotton cultivation. Fertilizer treatments were N as urea, P as monoammonium phosphate (MAP), and K as potassium chloride. The nitrification inhibitor, (PO) was added to urea and MAP at the rate of 2%. Two treatments: N₁₅₀P₁₄₀K₆₀ (T₁) and N₁₅₀P₁₄₀K₆₀ (T₂) (subscripts are concentrations in mg kg⁻¹ soil) were applied. The control (C) was without fertilizer and PO. Populations of oligotrophic bacteria, ammonifying bacteria, nitrifying bacteria, denitrifying bacteria, mineral assimilating bacteria, oligonitrophic bacteria, and Azotobacter were determined by the most probable number method. Treatment T2 increased the number of oligonitrophic bacteria, utilization of mineral forms of nitrogen by ammonifying bacteria, decreased the number of nitrifying bacteria, denitrifying bacteria and net nitrification, and increased the cellulose degradation activity of soil. In conclusion, our experiment showed that PO combined with mineral fertilizer is a most promising compound for inhibiting nitrification, which increased N fertilizer availability and efficiency to the cotton plants.

KEYWORDS
Urea, MAP, nitrification inhibitor, cellulose decomposition, microorganisms

INTRODUCTION
Nitrogen fertilizers in the form of urea are commonly applied in Uzbekistan in order to increase cotton yield in low fertile soils. The NO₃ formed through nitrification of urea is susceptible to loss by leaching and may contribute to NO₃ pollution of ground- and surface waters. Treatments of fertilizers with nitrification inhibitors have been suggested as a technique to reduce the nitrification rate and NH₃ volatilization (Malzer, 1979; Malhi and Nylor, 1982; McCarty and Bremner, 1990; Freney et al., 1992). Nitrification inhibitors may potentially reduce NO₃ losses by leaching from NH₄-N, liberating fertilizer materials, including organic N sources, by maintaining N as NH₄⁺, which is less susceptible to loss from the soil by this route, and NH₃ volatilization (Bremner and Krogmeier, 1989; Smith and Hadley, 1992; Poberejskaya et al., 1993; Kholdebarin et al., 1998). The soil microorganisms are thus of great importance to the nitrogen nutrition of the crop vegetation. They are sensitive to changes in the surrounding soil (Hodges, 1990; Schinner and Sonnleitner, 1996). It has been shown that the microbial population changes after fertilization (Hyman et al., 1990; Dobbs, 1992; Anonymous, 1992). Fertilizer can directly stimulate the growth of microbial populations as a whole by supplying nutrients and may affect the composition of individual microbial communities in the soil (Khonje et al., 1989; Sarathchandra et al., 1989; Khamis et al., 1990).

The effects of the nitrification inhibitor, Potassium oxalate, with a combination of fertilizers (200kg ha⁻¹) on the soil microbial population has been studied in our previous work experiments. In this study we reduced the amount of fertilizer combined with Potassium oxalate. The purpose of this study was to investigate the influence of mineral fertilizer (150 kg ha⁻¹) combined with potassium oxalate on the soil microbial population and the activities and nitrification rate in nitrogen deficient calcareous soil Uzbekistan under cotton cultivation.
MATERIALS AND METHODS

STUDY SITE AND SOIL SAMPLING

Sites used in this study represent continuously cultivated (more than 50 years) cotton fields located in Kalinin province, the northeastern part of Uzbekistan. The soil type is calcareous Calcosol having a calcic horizon within 80 cm of the surface. The orchic horizon is low in organic matter. The climate is semi-arid with mean annual air temperatures of 16°C and 18°C, and mean annual rainfalls of 200 mm. Soil samples were taken from the top 10 cm of soil from an existing cotton field. The cores were pooled and field-moist soils were sieved (<2mm) directly after collection. The soil samples were kept in black polyethylene bags and stored at 4°C. These “fresh” field-moist, sieved samples were used for the incubation study.

POT EXPERIMENTS

Soil microbial activity and N transformation in soils amended with the mineral fertilizers and combined with potassium oxalate were studied in small pots in laboratory experiments with three replicates. Field-moist sub samples (1kg) of each treatment replicate were placed in pots and treated with N as Urea at a rate of 150 mg kg⁻¹ soil. P was supplied as MAP at a rate of 140 mg P kg⁻¹ soil and Potassium chloride at a rate of 60 mg K kg⁻¹ soil. PO was added to urea and MAP at a rate of 2%. The control pots (N₀P₀K₀) received neither PO nor fertilizers. Two treatments: N₁₅₀P₁₄₀K₀(T₁) and N₁₅₀P₀K₁₄₀(T₂) were applied for this study. The tested pots were then placed in incubators maintained at 27°C for 45 days.

SOIL CHEMICAL AND PHYSICAL ANALYSIS

Air-dried samples were analyzed for the total C, N, P, K and Mg contents. Soil particle distribution was determined using sodium phosphate. The soil chemical and physical properties are presented in Table 1. The total carbon content, Cₜot, was identified by elementary analysis while total nitrogen, Nₜot, content was determined by the Kjeldahl method. The molybdenum blue method determined the total phosphorus content, Pₜot, in soil. Potassium, K, was determined using the Flame Photometric Method (Riehm, 1985). The Atomic Absorption Spectrophotometer (AAS) was employed to measure calcium chloride (CaCl₂) and extractable magnesium (Schachtahnabel and Heinemann, 1974). Soil pH-value was measured by means of electrometer.

SOIL MICROBIOLOGICAL ANALYSES

After 45 days pots were removed from the incubation and were analysed for microbiologic tests. A plate dilution method was used for the determination of numerous microorganisms using agar medium. In order to count the number of microorganisms, 10 g of soil was shaken with 90 ml of sterilized distilled water. From this suspension the serial dilution (1:10) was prepared and plate counts were performed in triplicate and incubated until growth occurred (usually 3-7 days). CFU of ammonifying bacteria were enumerated on glycerine peptone agar. Mediums containing 10 g of starch, 2 g of (NH₄)₂SO₄, 1 g of K₂HPO₄, 1 g of MgSO₄ and 3 g CaCO₃, 1 g of NaCl and 15 g of agar 1⁻¹ were used for mineral assimilating bacteria. Nitrifying bacteria were determined on plates containing 2 g of (NH₄)₂SO₄, 1 g of K₂HPO₄, 0.5 g of MgSO₄, 0.1 g FeSO₄, 5 g CaCO₃, and 0.4 g NaCl⁻¹ of liquid medium. Denitrifying bacteria on Gilby medium containing 1 g KNO₃, 1g KH₂PO₄, 1g K₂HPO₄, 2 g MgSO₄, 0.2 g CaCl₂, 0.1 mg FeCl₃, 0.1% solution of brom thymolblue, oligotrophic bacteria on soil agar containing 900 ml water, 100g soil, 18g agar L⁻¹, oligotrophic bacteria and Azotobacter were determined on Eshbi agar containing 0.2 g K₂HPO₄, 0.2 g MgSO₄, 0.2 g of NaCl, 0.1 g K₂SO₄, 5 g CaCl₂, 20 g sacharosa, and agar 15 g 1⁻¹.

SOIL BIOCHEMICAL MEASUREMENTS

Cellulose degrading activity of soil was measured according to Suyaginzew (1987). Cellulose material was placed into soil for an incubation period of 45 days. After 45 days the material was removed and the cellulose degradation percentage was analyzed. Net nitrifications were measured by incubating the soil samples with the soil moisture content adjusted to 60% of the WHC at 28°C for 45 days. The method used is described in detail in Aristowskaya (1962). The data were analyzed using the statistical analysis of variance by Tepper (1974).

RESULTS AND DISCUSSION

Changes in soil microbial populations

T1 and T2 decreased the number of oligotrophic bacteria compared to the control (Table 2). A decreasing of coloniza-

### Table 1. Chemical and physical parameters for the 0-30cm depth.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Physical</th>
</tr>
</thead>
<tbody>
<tr>
<td>C𝑡ot</td>
<td>mg 100g⁻¹</td>
</tr>
<tr>
<td>200</td>
<td>.6</td>
</tr>
</tbody>
</table>
Table 2. Effect of mineral fertilizer combined with potassium oxalate (PO) on the number of oligotrophic, ammonifying and mineral assimilating bacteria (10^6 cfu g^-1 soil)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oligotrophic</th>
<th>Ammonifying</th>
<th>Mineral assimilating</th>
</tr>
</thead>
<tbody>
<tr>
<td>N_0P_0K_0</td>
<td>184.0 ± 0.62</td>
<td>7.7 ± 0.25</td>
<td>94.7 ± 0.59</td>
</tr>
<tr>
<td>N_150P_140K_60</td>
<td>10.6 ± 0.51</td>
<td>8.9 ± 0.55</td>
<td>27.4 ± 0.62</td>
</tr>
<tr>
<td>N_150P_0P_140K_60</td>
<td>50.0 ± 0.77</td>
<td>4.5 ± 0.47</td>
<td>17.2 ± 0.83</td>
</tr>
</tbody>
</table>

Table 3. Effect of mineral fertilizer combined with potassium oxalate (PO) on the number of oligonitrophilic denitrifying and nitrifying bacteria (10^6 cfu g^-1 soil)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oligonitrophilic</th>
<th>Denitrifying</th>
<th>Nitrifying</th>
</tr>
</thead>
<tbody>
<tr>
<td>N_0P_0K_0</td>
<td>2.6 ± 0.90</td>
<td>700.0 ± 0.59</td>
<td>0.110 ± 0.79</td>
</tr>
<tr>
<td>N_150P_140K_60</td>
<td>12.7 ± 0.67</td>
<td>70.0 ± 0.65</td>
<td>0.25 ± 0.49</td>
</tr>
<tr>
<td>N_150P_0P_140K_60</td>
<td>7.3 ± 0.59</td>
<td>60.0 ± 0.38</td>
<td>0.13 ± 0.55</td>
</tr>
</tbody>
</table>
Table 4. Effect of mineral fertilizer combined with Potassium oxalate (PO) on the net nitrification and cellulose degradation activity of soil.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Net nitrification</th>
<th>Cellulose degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg N-NO₃</td>
<td>% % % % %</td>
</tr>
<tr>
<td>PO</td>
<td>2.15 ± 0.72</td>
<td>0.8 ± 0.85</td>
</tr>
<tr>
<td>P₅ K₀</td>
<td>12.15 ± 1.14</td>
<td>12.3 ± 1.04</td>
</tr>
<tr>
<td>P₁₄₀ K₆₀</td>
<td>6.98 ± 1.00</td>
<td>3.75 ± 1.98</td>
</tr>
</tbody>
</table>

CONCLUSIONS
It was clearly demonstrated that fertilization supplied with nitrification inhibitors influenced soil microorganisms. All combinations of mineral fertilizers combined with PO during the incubation had an inhibitory effect on the activity of oligotrophic bacteria, ammonifying bacteria, and denitrifying bacteria. The marked stimulus effect on the number of bacteria during the incubation was achieved with T2 and the lowest with T1. To summarize, the work reported in this paper suggests that PO combined with mineral fertilizers had no adverse effects on the biological nitrogen fixing bacteria Azotobacter and increased the activity of oligotrophic philic bacteria and increased the cellulose degrading activity in soil. PO indicated potential as nitrification inhibitors for the soil of urea used in this study. The treatment T2 decreased the net nitrification compared with fertilizer alone. In conclusion PO is one of the promising nitrification inhibitor compounds for reducing potential NO₃ leaching losses by nitrifying microorganisms from materials during cotton plant establishment.

LITERATURE CITED
Anonymous, K. 1992. Fish fertilizer has proved soil stimulant. Straight Furrow 47:7